## Remarks

Claims 33-99 having been canceled, the pending claims are claims 1-32.

Request to Correct Inventorship Pursuant to 37 C.F.R. §1.48(b)

It is respectfully submitted that prosecution of the above-identified patent application has resulted in an election of claims, pursuant to a Restriction Requirement, such that fewer than all of the currently named inventors are the actual inventors of the invention presently under examination.

The following individuals were named inventors of the above-identified patent application according to a Declaration and Power of Attorney submitted October 3, 2001: Fred E. Regnier, Xiang Zhang and Asish Chakraborty. However only Fred E. Regnier is an inventor of the subject matter of claims 1-32. The nonelected claims (claims 33-99) have been canceled in view of Applicants' election of Group I (claims 1-32) pursuant to the Restriction Requirement mailed May 7, 2002. As the invention of Xiang Zhang and Asish Chakraborty is no longer being claimed in the application, it is respectfully requested that the application be amended in accordance with 37 C.F.R. §1.48(b) to delete Xiang Zhang and Asish Chakraborty as named inventors.

OK to enter PP 6/16/03

A check for the processing fee in the amount of $130.00 is attached for the correction of inventorship, in accordance with 37 C.F.R. §§1.48(b)(2) and 1.17(i). The Assistant Commissioner is hereby authorized to charge any additional fees required for this petition or credit any overpayment to deposit Account No. 13-4895.

Rejection under 35 U.S.C. §112, Second Paragraph

The Examiner rejected claims 1-32 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. This rejection is respectfully traversed.

The Examiner objected to the use of the term "isotopic variant" in claims 1, 4, 5 and 6 as vague and indefinite because it is unclear from what bases the variation is derived. Applicant disagrees. Claims 1, 4, 5 and 6 recite an "isotopic variant *of a chemical moiety*" (emphasis added). This recitation in the claims themselves clearly shows that the "chemical moiety" serves as the basis from which the variation is derived. Isotopic variants of a chemical moiety would be compounds that are chemically identical (i.e., the same chemical moiety) but isotopically different (i.e., containing different mass isotopes). For further support, and to exemplify "isotopic variants", the Examiner's attention is directed to the specification at page 10, lines 8-12, wherein it is stated:

> The labeled proteins, peptides or peptide cleavage products are isotopically distinct because they contain different isotopic variants of the same chemical entity (e.g, a peptide in the first sample contains $^{1}H$ where the peptide in the second sample contains $^{2}H$; or a peptide in the first sample contains $^{12}C$ where the peptide in the second sample contains $^{13}C$).

It is respectfully submitted that the use of the term "isotopic variant" in claims 1, 4, 5 and 6 does not render the claims indefinite.

The Examiner also objected to the use of the term "normalized isotope ratio" in claims 1, 3, 5 and 6, because it is unclear as to what the isotope ratio is being compared to render it "normalized." Applicant disagrees. The claims recite "a *normalized isotope ratio characterizing proteins whose concentration is the same in the first and second samples*." The claims further recite "*an isotope ratio of the first and second isotopically labeled proteins*" and go on to state that "a difference in the isotope ratio of the first and second isotopically labeled proteins and the normalized isotope ratio is indicative of a difference in concentration of the protein in the first and second samples." Applicants maintain that it is clear from the claim language itself that the term "normalized" is used to identify the isotope ratio (i.e., mass spectrometry signal in first sample vs. mass spectrometry signal in second sample) of proteins whose concentration remains unchanged. This normalized isotope ratio becomes the standard against which the isotope ratio (i.e., mass spectrometry signal in first sample vs. mass spectrometry signal in second sample) of

the first and second isotopically labeled proteins is compared in order to determine whether their concentrations are different in the first and second samples.

At page 36, line 23, bridging to page 37, line 4, the specification elaborates on this concept:

> In a complex combined mixture, there may be hundreds to thousands of peptides, and many of them will not change in concentration between the control and experimental samples. These peptides whose levels are unchanged are used to establish the normalized isotope ratio for peptides that were neither up nor down regulated. All peptides in which the isotope ratio exceeds this value are up regulated. In contrast, those in which the ratio decreases are down regulated. A difference in relative isotope ratio of a peptide pair, compared to peptide pairs derived from proteins that did not change in concentration, thus signals a protein whose expression level did change between the control and experimental samples. If the peptide characterized by an isotope ratio different from the normalized ratio is a signature peptide, this peptide can be used according to the method of the invention to identify the protein from which it was derived.

It is respectfully submitted that is it clear that the term "normalized isotope ratio" in claims 1, 3, 5 and 6 describes the ratio of mass spectrometry signals in first and second samples for proteins whose concentration has not changed, and that the use of this term does not render the claims indefinite.

Reconsideration and withdrawal of the rejection of claims 1-32 under 35 U.S.C. §112, second paragraph, is respectfully requested.

Rejection under 35 U.S.C. §102

The Examiner rejected claims 1-4 and 6 under 35 U.S.C. §102(a) as being anticipated by Chen et al. (*Anal. Chem.* 2000;*72*(6):1134-1143). This rejection is respectfully traversed.

Claims 1-4 and 6 are directed to a method for determining a *difference in the concentration* of a protein present (or originally present, in the case of claim 6) in first and

second samples relative to other proteins present in both samples. Applicant's invention, as exemplified by claim 1, includes the following steps:

covalently attaching a first isotopic variant of a chemical moiety to a protein in the first sample to yield at least one first isotopically labeled protein;

covalently attaching a second isotopic variant of the chemical moiety to a protein in the second sample to yield at least one second isotopically labeled protein, wherein the first and second isotopically labeled proteins are chemically equivalent yet isotopically distinct;

mixing at least portions of the first and second samples to yield a combined sample; and

subjecting the combined sample to mass spectrometric analysis to determine a normalized isotope ratio characterizing proteins whose concentration is the same in the first and second samples and an isotope ratio of the first and second isotopically labeled proteins, wherein a difference in the isotope ratio of the first and second isotopically labeled proteins and the normalized isotope ratio is indicative of a difference in concentration of the protein in the first and second samples.

Optionally, the proteins are fragmented before (e.g., claims 5 and 6) or after (e.g., claim 4) isotopic labeling.

Chen et al., in clear contrast, teach nothing more than a simple protein *detection* method. Chen et al. produced isotopically labeled proteins in cell culture by supplying deuterated amino acid precursors (glycine or methionine) during protein biosynthesis. The Chen et al. system involves inducible expression of ubiquitin from an expression vector. Three basic experiments are described in Chen et al., *none of which involve evaluating the difference in relative concentration of proteins in two different samples.*

In a first experiment (see "Tryptic Digestion and MALDI MS Analysis," page 1136, first column) Chen et al. teach isolation of His-tagged ubiquitin, optional mixing of purified, labeled and unlabeled ubiquitin, tryptic digestion of the combined sample or the individual samples, and mass spectrometric (MS) analysis of the tryptic peptides (Fig. 2). Tryptic peptides are identified by mass and sequence (Table 1). MS of the tryptic peptides in the combined sample shows the

expected mass splitting. The purified samples subjected to tryptic digest do *not* contain proteins other than ubiquitin, thus a *normalized isotope ratio*, as recited in claim 1, *is not (and could not be) determined.* This precludes determination of a difference in isotope ratio between first and second isotopically labeled proteins and the normalized isotope ratio in accordance with claim 1, which difference is indicative of a difference in concentration of the protein in the first and second samples.

In a second experiment (see "Mass Tagging in *E. coli* Cells and the Target Protein Identification," page 1136, first column, and "Identification of UBL1 in an *E. coli* Extract," page 1139, second column) Chen et al. perform MS analysis of only one sample, i.e., the cell extract from a cell culture grown in media supplemented with 50% labeled amino acid precursors. The presence of ubiquitin is confirmed by observation of the expected tryptic peptides and the expected mass splitting. *Two samples are not taught, so protein concentration changes cannot be detected* as recited in claim 1.

A third experiment (see "Mass Tagging for a Complex Mixture and MALDI MS Analysis, page 1136, first column, and page 1139, second column) involves MS analysis of an isolated complex of ubiquitin and ubiquitin conjugating enzyme. Cell cultures were supplied with a mixture of amino acids that included 50% deuterated glycine. The peak pairs and mass splits expected for the components of the complex were observed. As in the first experiment, the samples were purified so the *concept of a normalized isotope ratio has no meaning* in this experiment. Further, it appears that *only one sample* was analyzed, and *differences in protein concentration were not evaluated.*

Applicant submits that the method of claims 1-4 and 6 is patentably distinct from the method disclosed in Chen et al. for at least the foregoing reasons. In addition, the method of claim 6 recites that the proteins are fragmented prior to isotopic labeling. Chen et al. describes only an intracellular biosynthetic labeling process, and does not teach isotopic labeling subsequent to protein fragmentation. It is accordingly respectfully submitted that Chen at al. do not teach every element of the claimed invention, as required to sustain a rejection under 35

U.S.C. 102. Reconsideration and withdrawal of the rejection of claims 1-4 and 6 as being anticipated by Chen et al. (*Anal. Chem.* 2000;*72*(6):1134-1143) under 35 U.S.C. 102(a) is respectfully requested.

The Examiner rejected claims 1-32 under 35 U.S.C. §102(e) as being anticipated by Geng et al. (*Journal of Chromatography A*, 2000; *870*(1-2):295-313). This rejection is respectfully traversed.

To begin with, Applicant points out that this rejection is improper in that the cited document is not a patent or application for patent. Applicant will proceed on the basis that this was an oversight and the Examiner intended to make this rejection under 35 U.S.C. §102(a). If, however, this is an incorrect assumption, Applicant requests clarification and a full opportunity to address the rejection, if maintained.

Applicant Fred E. Regnier is a the sole inventor of the claims elected and presently under examination in the present application. Applicant Regnier is also a co-author of Geng et al. The other two co-authors, Minghui Geng and Junyan Ji, are not inventors of the subject matter of the elected claims or of information that is commonly disclosed in the present application and in the Geng et al. document. In this regard please see the Declaration under 37 U.S.C. §1.132 of Fred E. Regnier, Ph.D., submitted herewith. In view of the Declaration of Dr. Regnier and the above comments, it is respectfully requested that Geng et al. be removed as a prior art reference pursuant to MPEP 715.01(c). Reconsideration and withdrawal of the rejection of claims 1-32 under 35 U.S.C. §102(e) or (a) as being anticipated by Geng et al. (*Journal of Chromatography A*, 2000; *870*(1-2):295-313) is respectfully requested.

Information Disclosure Statement mailed January 11, 2002

In a telephone message left with Applicant's Representative Victoria Sandberg on March 14, 2003, the Examiner indicated that the documents mailed with the Information Disclosure Statement on January 11, 2002, could still not be found.

In accordance with the Examiner's Request, Applicant has enclosed for the Examiner's convenience replacement copies of the "Other Documents" listed on the 1449 form and which have not yet been considered by the Examiner. Also attached, as Exhibit A, is a copy of the Information Disclosure Statement and the 1449 form(s) mailed January 11, 2002, along with a date stamped postcard indicating that the United States Patent and Trademark Office did receive all the copies of the documents originally submitted.

Consideration of the "Other Documents" listed on the 1449 form(s) mailed January 11, 2002, and return of an initialed copy of the 1449 form(s) with the next Official Communication, is respectfully requested. Should any of the cited documents form the basis of a rejection of the claims, Applicant kindly requests that the next Office Action be made non-final to afford the Applicant a full opportunity to respond to the rejection, insofar as these documents were in fact originally provided to the USPTO prior to substantive examination in this matter.

## Sequence Listing

In accordance with the requirements of 37 C.F.R. §1.821 et seq., Applicants submit written and computer readable forms of a Sequence Listing. The information recorded in computer readable form is identical to the written Sequence Listing. Furthermore, it is submitted that the Sequence Listing contains no new matter.

## Summary

It is respectfully submitted that the pending claims 1-32 are in condition for allowance, and notification to that effect is earnestly requested. The Examiner is invited to contact Applicants' Representatives, at the below-listed telephone number, if it is believed that prosecution of this application may be in any way accelerated or assisted thereby.

Respectfully submitted for
Fred E. Regnier

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26813
PATENT TRADEMARK OFFICE

March 24, 2003
Date

By: _____
Victoria A. Sandberg
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CERTIFICATE UNDER 37 CFR §1.10:
"Express Mail" mailing label number: EV153782770US Date of Deposit: 24 March 2003
The undersigned hereby certifies that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR §1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

By: _____
Name: JACQUELYN K TORBERG

Exhibit A

PATENT
Docket No. 290.0001 0101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



Applicant(s): Fred E. REGNIER et al.
Serial No.: 09/849,924
Confirmation No.: 8955
Filed: 4 May 2001

Group Art Unit: 1645
Examiner: Unassigned

For: AFFINITY SELECTED SIGNATURE PEPTIDES FOR PROTEIN IDENTIFICATION AND QUANTIFICATION

## INFORMATION DISCLOSURE STATEMENT



Assistant Commissioner for Patents
Washington D.C. 20231

Sir:

In compliance with the duty imposed by 37 C.F.R. § 1.56, and in accordance with C.F.R. §§ 1.97 *et. seq.*, the materials enclosed herewith are brought to the attention of the Examiner as possibly being of interest in connection with the above-identified patent application. Consideration of each of the documents listed on the attached 1449 forms is respectfully requested. Pursuant to the provisions of M.P.E.P. §609, Applicants further request that a copy of the 1449 forms, marked as being considered and initialed by the Examiner, be returned with the next Official Communication.

Applicants also wish to bring the Examiner's attention to the following pending U.S. provisional application, as well as any prior art referenced therein. A copy of the below-listed pending U.S. provisional Patent Application is provided herewith.

### List of Pending Non-Published U.S. Patent Applications

| Applicant(s) | Application Number | Filing Date | Serial No. of any Provisional Application to which listed Application claims priority |
|---|---|---|---|
| Zhang et al. | 60/325,335 | 09/27/01 | |

It is believed that no fee is due, as this Information Disclosure Statement is filed prior to the receipt of any Action on the merits. However, in the event a fee is due, please charge any fee or credit any overpayment to Account No. 13-4895.

The Examiner is invited to contact Applicants' Representatives at the below-listed telephone number, if they can be of any assistance during prosecution of the present application.

Respectfully submitted for



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CERTIFICATE UNDER 37 C.F.R. 1.8:

The undersigned hereby certifies that this paper is being deposited in the United States Postal Service, as first class mail, in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on this 11 day of January, 2002.

David L. Provence

January 11, 2002
Date

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